The Role of Cholesteryl Ester Transfer Protein in Primate Apolipoprotein A-I Metabolism

Insights From Studies With Transgenic Mice

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To assess the effects of cholesteryl ester transfer protein (CETP) on the primate lipoprotein profile, a transgenic mouse expressing cynomolgus monkey CETP was developed. The C57BL/6 mouse was used, and four lines expressing the primate CETP were established. The level of CETP activity in the plasma of the transgenic mice ranged from values similar to those obtained for the monkey to levels approximately sixfold higher than that in the normal monkey. When all of the lines were taken into consideration, there was a strong \((r=-0.81\) or higher, \(p<0.01\)) negative correlation between plasma CETP activity and total plasma cholesterol, plasma apo A-I levels, and plasma apo A-I to apo B ratio. There was a strong positive correlation \((r=0.77)\) between plasma CETP activity and plasma apo B levels. The size of the apo A-I-containing lipoproteins was significantly reduced in mice with high plasma CETP activity, and that reduction in size was due to the absence of the larger (HDL, and HDL,) apo A-I-containing particles in the plasma. When the transgenic mice were fed a high-fat, high-cholesterol diet, the effects of the diet on lipoprotein profile were more prominent in the CETP transgenic mice than the controls. The CETP transgenic mice had, for example, substantially higher plasma cholesterol and plasma apo B levels \((p<0.01)\), and the apo B-containing lipoproteins were generally larger than those in the nontransgenic C57BL/6 mice consuming the same diet. These studies show that expression of primate CETP in the C57BL/6 mouse has a marked effect on the mouse's apo B- and apo A-I-containing lipoproteins, and they establish a metabolic basis for the previously reported (Arterioscler Thromb 1991;11:1759-1771) negative correlation between hepatic CETP mRNA levels and plasma apo A-I levels in the cynomolgus monkey. In addition, they suggest that increased CETP activity is associated with an atherogenic shift in the lipoprotein profile. (Arteriosclerosis and Thrombosis 1992;12:736-744)

KEY WORDS • cholesteryl ester transfer protein • apolipoprotein A-I • apolipoprotein B • high density lipoproteins • cholesterol • atherogenic diet • transgenic mice

High density lipoproteins (HDLs), because they can carry cholesterol into and out of the interstitium,\(^1\) are believed to play a key role in the interchange of cholesterol between the plasma and the tissues. In addition, because of the strong negative correlation between plasma HDL cholesterol levels and the incidence of coronary artery disease,\(^2-6\) it is thought that perturbations in the metabolism of HDL may significantly influence the atherogenic process. That is especially true in experimental atherosclerosis. In most models of that disease, the diet-induced changes in the lipoprotein profile include not only a marked increase of the apolipoprotein (apo) B-containing lipoproteins, but also a severe reduction of the HDL levels. Although early studies focused primarily on the increased levels of the apo B-containing lipoproteins, it is now becoming clear that these diet-induced alterations in HDL metabolism are also contributing significantly to the development of the disease. An example of the magnitude of the effect that alterations of HDL metabolism can have on the development of the experimental disease is evident from studies of diet-induced atherosclerosis in different inbred strains of mice.\(^7,8\) The key difference noted in those studies between the "susceptible" and "resistant" strains was the degree to which the HDL levels were reduced in response to the atherogenic diet, i.e., those mice able to maintain their plasma levels of HDL tended to be resistant to lesion development, while those whose plasma HDL levels decreased developed relatively complex lesions. None of the difference in frequency or severity could be explained by differences in the low density lipoprotein (LDL) levels. Subsequent studies using apo A-I transgenic mice\(^9\) have tended to confirm this potent influence of HDL on lesion progression. Thus, HDL is strongly implicated as a factor in the experimental disease, and a clear understanding of the genes that regulate HDL metabolism should provide insights that might allow pharmacological manipulation of plasma HDL levels and possibly the rate at which the experimental disease progresses.

We have examined the effect of the atherogenic diet on HDL metabolism in the nonhuman primate.\(^10-14\) Those studies showed that 1) plasma HDL cholesterol and apo A-I concentrations were reduced in response to...
Animals and Diets

Production of Transgenic Animals

C57BL/6 mice were obtained from the Upjohn mouse colony and fed Purina mouse chow (Ralston Purina Co.) unless indicated otherwise. That diet contained (by weight) 4.5% animal fat and <0.03% cholesterol. In some studies a special high-fat, high-cholesterol diet was used to provide a polyadenylation site. The transgene was injected into the pronuclei of C57BL/6 fertilized eggs. CETP, cholesteryl ester transfer protein.

Methods

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Production of Transgenic Animals

CETP transgenic mice were generated by pronuclear microinjection of C57BL/6 UPJ (BR) eggs that had been fertilized by the same strain. The protocol for production of the transgenic mice was a modification of the methods described by Brinster et al and Hogan et al. The pronuclei of oocytes from the C57BL/6 UPJ (BR) mice were injected with a DNA construct contain...
beled cDNA to determine whether any rearrangements of the transgene had occurred.

RNA was isolated from tissues of nonfasted animals by homogenizing the tissues in RINzol and following the protocol supplied by the manufacturer (CINNA BIOTEX). RNA preparations were digested with RNase-free DNase, phenol-chloroform extracted, and ethanol precipitated. RNA pellets were washed with 70% ethanol, dried, and dissolved in TE solution (10 mM Tris [pH 7.5] and 1 mM EDTA). RNA concentration was determined by absorbance at 260 nm. The tissues expressing the primate CETP were identified, and the mRNA concentration in those tissues was determined using the RNase protection assay described by Pape et al.24

Cholesterol and Lipoprotein Measurements

Blood samples (approximately 75 µl) were collected from the periorbital sinus into heparinized microhematocrit tubes from animals that had been fasted overnight. The plasma was obtained by centrifugation, diluted 1/10 with phosphate-buffered saline (PBS) containing EDTA (0.01%) and sodium azide (0.02%), and stored at 4°C until analyzed. The plasma cholesterol concentration was determined by enzymatic assay (Boehringer-Mannheim) as described by the manufacturer.

Apo A-I was purified from mouse plasma exactly as described previously for cynomolgus monkey apo A-I.25 Monospecific antibody to mouse apo A-I was prepared by injecting cynomolgus monkeys subcutaneously with 200 µg purified mouse apo A-I dissolved in complete Freund’s adjuvant. The anti-apo A-I was originally prepared for studies of mouse apo A-I metabolism in transgenic mice expressing cynomolgus monkey apo A-I, and therefore, monkeys were used because an antibody to mouse apo A-I was required that did not recognize primate apo A-I. The monkeys received booster injections at 1 and 2 months. The antibody so prepared was evaluated by Western immunoblotting using mouse serum and was found to recognize only proteins that comigrated with mouse apo A-I. The mouse apo B antibody was kindly provided by Dr. Beverly Paigen, The Jackson Laboratory, Bar Harbor, Me.

The plasma apo A-I and apo B concentrations were determined by electroimmunoassay essentially as described previously10,12,15 except that 1) the antisera concentration in the gels used for apo A-I measurements was 2% by volume, 2) they contained NP-40 (0.06%), and 3) the electrophoresis was run at 5 V/cm for 18 hours; the antisera concentration in the gels used for apo B measurements was 0.4% by volume, the gels contained polyethylene glycol 8000 (3%) instead of dextran, and the electrophoresis was run at 3 V/cm for 18 hours. In each instance various dilutions of mouse reference plasma were run with each set of unknowns, and the concentration of apo A-I or apo B in the sample was determined relative to the reference plasma. The apo A-I concentration (in milligrams per deciliter) of the reference plasma was determined by comparing the height of the rocket produced when mouse reference plasma was run in the assay with that of various dilutions of purified mouse apo A-I. The apo B concentration (in milligrams per deciliter) of the mouse reference plasma was determined exactly as described previously.15 Briefly, the apo B from the reference plasma was purified and its concentration determined by a modification of the method of Lowry et al.26 Apo B losses that occurred during the purification were corrected for by use of an internal standard added at the beginning of the purification process.12,15

Assay of CETP Activity

Cholesteryl ester transfer activity was determined as described previously44 using a modification of the procedure of Morton and Zilversmit.27 Human LDL (d=1.019–1.063 g/ml) radiolabeled with [4,14C]cholesteryl ester served as the donor, and human HDL (d=1.063–1.225 g/ml and purified by 4% agarose column chromatography14) was used as the acceptor.

High Density Lipoprotein and Low Density Lipoprotein Size Determinations

The size distribution of the apo B- and apo A-I-containing lipoproteins was determined by agarose column chromatography and nondenaturing gradient gel immunoblotting exactly as described previously.13 When plasma from individual mice was analyzed by column chromatography, 100 µl of a 1/10 dilution of the mouse plasma was applied to the column. When pooled samples were analyzed, 0.5–1.0 ml of mouse plasma was analyzed. In all instances the column buffer (PBS) contained bovine serum albumin (1 mg/ml) to prevent proteins in the mouse plasma from adsorbing to the column components.

Statistics

Plasma cholesterol, apo A-I, apo B, and the size (Stokes’ diameter) of the HDL were used as the dependent variables in a 2x2x2 factorial design.28 The independent factors included strain (C57BL/6 or CETP), diet (chow or HFHC), and sex (male or female). The analysis of variance results are contained in Tables 2 and 3. SAS9 was used for the numerical calculations. Correlation analysis (Figure 3) was performed as described by Snedecor and Cochran.30 In all instances, the null hypothesis was not rejected unless p<0.10.

Results

Four transgenic lines were identified that contained a heritable CETP transgene (Table 1). Note that each line expressed different levels of CETP activity, but expression within any one line was relatively constant. At no time was zinc used to induce the expression of CETP in any of the experiments described in this study. The distribution of the cynomolgus monkey CETP mRNA among the various tissues of the transgenic mice was determined, and results typical of those obtained are shown in Figure 2. Small amounts of the cynomolgus monkey CETP mRNA were present in all of the tissues examined except lung and spleen, but the only tissues showing significant levels of that mRNA were the liver, brain, and heart. The level of expression by the liver was the highest by far. No expression of the CETP mRNA was apparent in the tissues of nontransgenic animals.
Plasma Cholesterol and Lipoprotein Levels

To evaluate the relation between the level of CETP expression and plasma lipoprotein profile, the mean plasma cholesterol, apo B, and apo A-I concentrations, as well as the mean apo A-I to apo B ratio for each of the lines shown in Table 1, were plotted versus the corresponding mean plasma CETP activity. The results of those analyses are shown in Figure 3. Those data indicate that expression of monkey CETP had a profound effect on the plasma lipoprotein profile of the mouse. As plasma CETP activity increased, the plasma cholesterol and apo A-I levels decreased and the plasma apo B levels increased. Those changes were most evident when the plasma apo B to apo A-I ratio was plotted (Figure 3D). Thus, an increase in CETP gene expression would appear to be associated with an atherogenic shift in the lipoprotein profile of the mouse.

Effects of a High-Fat, High-Cholesterol Diet

The question then arose as to what effect CETP expression would have on the response of the mice to an HFHC diet. To answer that question, the transgenic mouse line expressing the highest level of CETP (UCTP-20) was divided into two groups with approximately equal numbers of males and females in each group, and they were fed either an HFHC challenge diet or mouse chow for 7 weeks. C57BL/6 mice, grouped and treated identically, were used as controls. At the end of 7 weeks all of the mice were bled, and the plasma cholesterol, apo A-I, and apo B concentrations were determined. The results of those analyses are contained in Table 2. Those data indicate that there was a statistically significant effect of the gene, the diet, and the animal’s sex on all of the measurements. In addition, there were also some diet-gene interactions. For example, when male C57BL/6 mice were switched to the HFHC diet, their plasma cholesterol concentrations increased an average of 62 mg/dl and their apo B concentrations increased an average of 14 mg/dl (an observation consistent with previous reports31), whereas when the CETP males were switched to that diet, their plasma cholesterol levels increased by almost 200 mg/dl and their apo B concentrations increased by 25 mg/dl. Thus, the gene not only affected the animals’ basal plasma cholesterol and apo B concentrations but also had a marked effect on the degree to which each changed in response to the HFHC dietary challenge.

In addition to the concentrations of the plasma lipoproteins, the effect of the gene and diet on the size distribution of the apo B- and apo A-I-containing lipoproteins was also evaluated. The effects of CETP expression and the HFHC diet on the size of the apo B-containing lipoproteins are shown in Figure 4. Those data indicate that the increase in apo B-containing lipoproteins evident in the chow-fed transgenic mice was not associated with major changes in the size distribution of those particles (Figure 4A). Rather, the increase in plasma apo B levels appeared to be due principally to an increase in the number of apo B-containing particles in the circulation.

By contrast, the HFHC diet caused a marked increase in the mean size as well as the size range of the apo B-containing particles in both the CETP transgenic mice and the control mice (Figure 4B); however, in general the apo B-containing lipoproteins in the CETP transgenic mice were larger than those of the C57BL/6 controls. Thus, there is the suggestion of a diet-gene interaction in this regard as well.

Figures 5 and 6 and Table 3 show the effects of CETP expression and the HFHC diet on the size of the apo A-I-containing particles. When analyzed by gradient gel electrophoresis-immunoblotting, the apo A-I particles from chow-fed C57BL/6 mice were invariably the largest and those from chow-fed CETP mice were invariably the smallest in Stokes’ diameter (Table 3 and

### Table 1. CETP Activity Levels in Four Lines of CETP Transgenic Mice

<table>
<thead>
<tr>
<th>Line</th>
<th>C57BL/6</th>
<th>UCTP-34</th>
<th>UCTP-45</th>
<th>UCTP-50</th>
<th>UCTP-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>1.5±0.3</td>
<td>2.7±0.2</td>
<td>6.0±0.2</td>
<td>6.3±0.2</td>
</tr>
<tr>
<td>(21)</td>
<td>(7)</td>
<td>(9)</td>
<td>(11)</td>
<td>(16)</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.8±0.3</td>
<td>2.6±0.2</td>
<td>4.1±0.3</td>
<td>5.6±0.4</td>
<td></td>
</tr>
<tr>
<td>(27)</td>
<td>(5)</td>
<td>(10)</td>
<td>(7)</td>
<td>(11)</td>
<td></td>
</tr>
</tbody>
</table>

*Cholesteryl ester transfer protein (CETP) activity is expressed in arbitrary units, where one unit is equivalent to the corresponding mean plasma CETP activity. Values are mean±SEM for each line. The number of animals in each group is shown in parentheses.
Figure 5). Thus, the CETP gene reduced not only the concentration of the apo A-I–containing lipoproteins but also their mean size. Interestingly, there appeared to be a divergent effect in the animals’ response to the diet when measured by its effect on the size of the apo A-I–containing particles, i.e., in the mice expressing CETP, the diet caused the apo A-I–containing particles to increase in size, whereas in the C57BL/6 control mice the diet caused those particles to decrease in size. Consequently, there was a clear diet–gene interaction evident in this regard.

To more carefully evaluate the differences in the size of the apo A-I–containing particles in the chow-fed mice, fresh plasma from C57BL/6 mice and mice expressing CETP was analyzed by agarose (10%) column chromatography. A comparison of the apo A-I elution profiles from each of those pools is shown in Figure 6. Those data indicate that the reduction in particle size was due principally to the disappearance of the larger HDL (HDL, and HDL₃) from the circulation. Thus, the mouse HDL would seem to be a suitable substrate for primate CETP.

**Discussion**

A principal goal of these studies was to show that introduction of primate CETP into the plasma of the mouse would produce alterations in the intrinsic apo A-I–containing lipoproteins similar to those seen in the monkey when expression of the CETP gene was stimulated and thereby establish the link between increased CETP gene expression and decreased plasma apo A-I levels in the cynomolgus monkey. In the present study, CETP transgenic mice were produced by incorporating the cDNA encoding cynomolgus monkey CETP into C57BL/6 mice. Four strains of transgenic mice were produced which expressed the gene (as measured by plasma CETP activity) to varying degrees. Characterization of the lipoprotein profiles in those mice showed that expression of the primate CETP gene in the mouse

**Table 2. Effects of a High-Fat, High-Cholesterol Diet on Plasma Cholesterol, Apolipoprotein B, and Apolipoprotein A-I in CETP Transgenic Mice**

<table>
<thead>
<tr>
<th></th>
<th>C57BL/6</th>
<th>CETP transgenic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chow</td>
<td>HFHC</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>101±5</td>
<td>163±2</td>
</tr>
<tr>
<td>Apo B</td>
<td>12±1</td>
<td>26±2</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>118±9</td>
<td>179±3</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>84±4</td>
<td>153±11</td>
</tr>
<tr>
<td>Apo B</td>
<td>14±1</td>
<td>31±2</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>98±7</td>
<td>80±4</td>
</tr>
</tbody>
</table>

Plasma fraction (probability >f)

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>Apo B</th>
<th>Apo A-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>0.058</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>Diet</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>0.002</td>
<td>0.027</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gene x diet</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>0.127</td>
</tr>
<tr>
<td>Gene x sex</td>
<td>0.217</td>
<td>0.501</td>
<td>0.063</td>
</tr>
<tr>
<td>Diet x sex</td>
<td>0.038</td>
<td>0.599</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CETP, cholesteryl ester transfer protein; HFHC, high-fat, high-cholesterol; ANOVA, analysis of variance. All values for cholesterol, apolipoprotein (apo) B, and apo A-I are in milligrams per deciliter of plasma (mean±SEM).
FIGURE 4. Chromatograms showing the size distribution of apolipoprotein (apo) B- and apo A-I-containing lipoproteins from mice from each of the diet–gene treatment groups shown in Table 2. Several of the mice from each group shown in Table 2 were bled, and the plasma was pooled by strain–diet group, then passed through a 2.6x100-cm column of Bio-Rad A15M agarose, and fractionated as described previously.11 The apo B- and apo A-I–containing lipoproteins were identified by immunoassay13 and plotted versus elution volume (Ve). The actual elution volume is expressed as a fraction of the volume at which the albumin in that sample eluted (Valb). Mean±SD albumin elution volume was 410±5 ml. Panel A shows chromatograms obtained when plasma from chow-fed mice was analyzed. Panel B shows chromatograms obtained when plasma from mice consuming the high-fat, high-cholesterol (HFHC) diet was analyzed. CETP, cholesteryl ester transfer protein; vol, volume.

was associated with marked decreases in the size and plasma concentration of the apo A-I–containing particles in those animals. Because the presence of the cynomolgus monkey CETP cDNA was the only known difference in the genetic composition of the CETP transgenic mice compared with the C57BL/6 controls, these data strongly imply that the changes in apo A-I levels and size distribution were due to the presence of CETP in the plasma of the transgenic mice. Consequently, these observations, when taken together with the results of previous studies,10-14 support the proposition that increased expression of the CETP gene in the hypercholesterolemic cynomolgus monkey is largely responsible for the reduction in the size, plasma residence time, and therefore, concentration of apo A-I–containing lipoproteins in those animals.

It has been shown previously32,33 that the plasma HDL levels were markedly increased in humans with a genetic defect resulting in the absence of CETP in the circulation, and thus the potential role of CETP in HDL metabolism was clearly established. However, there have been no reports before this one that demonstrated that increased CETP gene expression caused a reduction in the size and concentration of intrinsic apo A-I–containing particles. A recent report by Agellon et al34 did show that transgenic (C57BL/6J×CBA/J; F1) mice containing a hybrid minigene encoding the human CETP and linked to the mouse MT-1 promoter had lower HDL cholesterol levels and smaller HDL (as measured by lipid staining) when the mice were given zinc, but those investigators could detect no effect of the minigene on the animals' plasma apo A-I levels, regardless of treatment. (No measurements were made of the apo A-I particle size distribution.) Thus, one might infer from those data that the increased CETP activity caused a decrease in the cholesterol ester content and the size but not the number of apo A-I–containing particles.

Our data contrast with those of Agellon et al34 in this respect, for they clearly establish that the size and concentration of the intrinsic apo A-I–containing particles were reduced in C57BL/6 mice expressing the monkey CETP. The reasons for these discrepancies cannot be determined unequivocally because there were some significant differences in the experimental design of our respective studies. For example, Agellon et al34 used a hybrid mouse (C57BL/6J×CBA/J; F1), whereas we used an inbred strain (C57BL/6). In addition, Agellon et al34 administered zinc to their animals to induce changes in the expression of their minigene, whereas no zinc was used in our studies (previous experiments have indicated that zinc administration has
a significant effect on the lipoprotein profile of the C57BL/6 mouse and thereby complicates interpretation of the results in that model; G.W. Melchior and K.R. Marotti, unpublished results). Finally, our genetic construct was different in several respects from that of Agellon et al,34 the most notable being that we used the cynomolgus monkey CETP cDNA, whereas they used a combination of genomic and cDNA fragments of human CETP. However, a comparison of the nucleotide and amino acid sequences of the monkey and human CETP14 indicates that there are few differences between the two proteins (the homology between human and monkey amino acid sequences was >95%), and in those cases where the amino acids were different, the predicted effect on protein structure was trivial. Therefore, it would appear that the differences were not due to differences in the protein. Rather, the principal differences in the two studies appear to be in the plasma CETP activity levels produced in the CETP transgenic mice. For example, the plasma CETP activity in the CETP transgenic mice of Agellon et al34 ranged from the same as to approximately 2.5-fold higher than that of their human reference plasma, whereas the plasma CETP activity in the CETP mice used in the present study ranged as high as sixfold that of the monkey reference plasma. Furthermore, in general we have found that CETP activity in the plasma of cynomolgus monkeys is more than two times higher than that of the human samples that we have analyzed. Therefore, the differences in the respective conclusions of the two studies are probably due primarily to differences in the levels of CETP activity in the plasma of the respective transgenic mice. Nonetheless, when taken together, the results of the two studies indicate that increases in CETP activity are associated with a reduction in plasma HDL levels, whether measured as a decrease in the lipid or the protein compo-

Table 3. Effect of CETP Expression and a High-Fat, High-Cholesterol Diet on the Size of Apolipoprotein A-I-Containing Lipoproteins as Measured by Gradient Gel Electrophoresis–Immunoblotting

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Diet</th>
<th>n</th>
<th>Stokes' diam (nm)</th>
<th>Mol wt (kd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>Chow</td>
<td>4</td>
<td>10.0±0.2</td>
<td>252±12</td>
</tr>
<tr>
<td>CETP</td>
<td>Chow</td>
<td>4</td>
<td>9.1±0.1</td>
<td>197±5</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>HFHC</td>
<td>4</td>
<td>9.7±0.1</td>
<td>231±7</td>
</tr>
<tr>
<td>CETP</td>
<td>HFHC</td>
<td>4</td>
<td>9.4±0.1</td>
<td>215±9</td>
</tr>
</tbody>
</table>

ANOVA factors

| Gene        | <0.001 |
| Diet        | 0.656  |
| Sex         | 0.503  |
| Gene×diet   | 0.002  |
| Gene×sex    | 0.684  |
| Diet×sex    | 0.181  |

HDL, high density lipoprotein; diam, diameter; mol wt, molecular weight; HFHC, high-fat, high-cholesterol; ANOVA, analysis of variance. Equal numbers of male and female mice were in each group. The cholesteryl ester transfer protein (CETP) transgenic mice were all from line UCTP-20. Values are mean±SD for each group.
nent. They further suggest that slight increases in the CETP activity affect HDL cholesterol content and size, but not the plasma apo A-I concentration, and that it is not unless the plasma CETP activity reaches levels comparable with that of the hypercholesterolemic monkey (2-4 A.U., Figure 3B) that a clear effect on the plasma apo A-I levels is detected.

It is not clear what effect, if any, the fact that mouse lipoproteins are the substrate for primate CETP has on our conclusions; however, it is noteworthy that the size and cholesterol content of the mouse HDL is very similar to that of the chow-fed cynomolgus monkey.

In addition to changes in the metabolism of the apo A-I-containing particles, these studies also showed that expression of cynomolgus monkey CETP in the mice had a profound effect on the animals' apo B metabolism. The concentration of apo B in the plasma of C57BL/6 mice used in these studies ranged between 10 and 20 mg/dl in animals fed the chow diet and between 20 and 35 mg/dl in those fed the HFHC diet. By contrast, the plasma apo B levels ranged between 20 and 25 mg/dl in the CETP mice fed the chow diet and between 40 and 50 mg/dl in CETP mice fed the HFHC diet. Thus, expression of cynomolgus monkey CETP in the mouse not only caused a distinct increase in the basal plasma apo B levels but also apparently made the mice substantially more reactive to the HFHC diet.

The mechanism by which CETP increased the plasma apo B levels cannot be deduced from these data; however, it is noteworthy that in cynomolgus monkeys fed the HFHC diet and in which the plasma CETP activity was increased two-to fourfold, the whole-body residence time of apo B was also significantly increased.\(^{15}\) Therefore, one could speculate that increasing CETP activity prolongs the time that apo B spends in the plasma. If that is in fact correct, it would not only explain the higher plasma apo B levels but also might explain why the mean size of the apo B-containing particles was larger in the CETP mice than in the C57BL/6 mice fed the HFHC diet, as a longer residence time might allow the CETP to transfer more cholesterol ester into the particle than might otherwise occur.

Finally, the question arises as to whether increased CETP activity is beneficial or deleterious with regard to the progression of atherosclerosis. One could argue, for example, that by increasing CETP activity, one stimulates the flow of cholesterol esters from the HDL to the apo B-containing lipoproteins, which are in turn cleared by the hepatic LDL receptors, i.e., that by stimulating CETP activity, one stimulates reverse cholesterol transport. Although these studies do not specifically address that question, they do indicate that increased CETP activity is associated with a much more atherogenic lipoprotein profile. For example, the apo A-I/apo B ratio averaged 9.8 in C57BL/6 male mice consuming the chow diet versus 3.7 in UCTP-20 male mice consuming that same diet (Table 2). Furthermore, when those same groups were fed the HFHC diet, that ratio decreased to only 6.9 in the C57BL/6 group, whereas it dropped to 3.2 in the UCTP-20 mice. That is noteworthy because although the females of this particular strain of mouse do develop arterial lesions when fed the HFHC diet, the males are generally more resistant to the diet-induced disease. However, by introducing the CETP gene into these males, we have altered their response to the atherogenic diet so that their apo A-I/apo B ratio is much closer to that of the atherosclerosis-susceptible C57BL/6 females fed that diet (that ratio averages 2.6 in the latter group; Table 2). Therefore, one might predict that the CETP transgenic male mice would have a greater propensity to develop arterial lesions when challenged with a high-fat diet than their nontransgenic counterparts. No studies specifically designed to test that hypothesis have been reported to date; however, if such is the case, it would indicate that CETP is an atherogenic protein and thus support the proposition\(^{14}\) that inhibition of its activity would inhibit the progression of atherosclerosis.

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References

31. Paigen B, Holmes PA, Mitchell D, Albee D: Comparison of atherosclerotic lesions and HDL lipid levels in male, female, and testosterone-treated female mice from strains C57BL/6, BALB/c, and C3H. Atherosclerosis 1987;64:215–221
The role of cholesteryl ester transfer protein in primate apolipoprotein A-I metabolism. Insights from studies with transgenic mice.
K R Marotti, C K Castle, R W Murray, E F Rehberg, H G Polites and G W Melchior

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